Low Prevalence of *GSC* Gene Mutations in a Large Cohort of Predominantly Caucasian Patients with Hidradenitis Suppurativa

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TO THE EDITOR

Hidradenitis suppurativa (HS) is a chronic dermatosis characterized by nodules and abscesses in apocrine gland–bearing sites. Mutations in three *GSC* genes have been identified in autosomal dominant forms of HS (Frew et al., 2017) (Supplementary Tables S1 and S2). *NCSTN, PSEN1,* and *PSENEN* mutations have been reported in 41 patients with unrelated HS, one Chinese family with HS, and 21 patients with unrelated HS with or without Dowling-Degos disease, respectively (Frew et al., 2017). The functional consequences of these mutations are yet to be elucidated. In the absence of segregation analysis and functional studies supporting their causality, it is possible that some reported variants are benign (Supplementary Table S2).

A total of three studies investigated *GSC* gene mutations in small patient cohorts (Supplementary Table S1). One of these studies explored *NCSTN, PSEN1,* and *PSENEN* in 48 British patients (Pink et al., 2012). A second study analyzed the six *GSC* genes (*NCSTN, PSEN1, PSENEN, PSEN2, APH1A,* and *APH1B)* in 20 patients from South Wales (Ingram et al., 2013). The third study investigated *NCSTN* in 95 Caucasian patients (Liu et al., 2016).

In contrast to our previous studies that focused on multiplex kindreds or on patients with syndromic HS and led to the identification of four *NCSTN* mutations (Duchatelet et al., 2019, 2015; Miskinyte et al., 2012; Sonbol et al., 2018), the recent study aimed at determining the prevalence of *GSC* gene mutations among a large cohort involving 169 unrelated patients who were mostly Caucasian with isolated or syndromic HS. This study was approved by the institutional review board (CPP Ile-de-France, Paris). Participants provided written informed consent and consented to the publication of their images. The characteristics of the cohort are detailed in Table 1 and Supplementary Table S3. We performed mutational analysis of the six *GSC* genes using a targeted next-generation sequencing gene panel, which also included *PSTP1P1* involved in a combination of pyoderma gangrenosum, acne, and HS and a combination of pyogenic arthritis, acne, pyoderma gangrenosum, and HS syndromes. Mutational analysis of the six *GSC* genes using a targeted next-generation sequencing gene panel also included *FGFR2* and *POFUT1* possibly implicated in HS (Calderón-Castrat et al., 2016; González-Villanueva et al., 2018; Higgins et al., 2017).

We identified two unreported *NCSTN* (NM_015331) mutations, p.Gly61Val (c.182G>T) and p.Gly576Val (c.1727G>T), in two unrelated familial cases (patients 111 and 150), predicted to be damaging by computational tools (Supplementary Table S4). The first mutation cosegregated with HS in the family (Figure 1 and Supplementary Figure S1). The proband's daughter carrying the familial mutation had an atypical presentation with multiple comedones outside HS predilection sites, suggesting the involvement of modifier genes and epigenetic and/or environmental factors. Cosegregation of the second mutation with the disease was not studied, as no additional family member was available. We also identified a previously reported *NCTSN* variant, c.996+7G>A, in a sporadic patient (patient 2) (Pink et al., 2012). However, this variant was inherited from the unaffected father and the study of the subject's keratinocytes mRNA did not detect *NCSTN* splicing nor expression anomaly, suggesting it was a nondeleterious change. In a familial HS case (patient 30), we identified a previously unreported *PSEN1* (NM_000021) mutation p.Ser390Glufs*20 (c.1167_1168insGA), inherited from the mother with Crohn's disease and spondylarthropathy (Figure 1 and Supplementary Figure S1). We found *PSENEN* (NM_172341) mutations in five patients with HS with Dowling-Degos disease (Figure 1 and Supplementary Figure S1). In patient 157, we identified the previously reported c.168T>G p.Tyr56* Jewish Ashkenazi founder mutation inherited from the mother with Jewish Ashkenazi ancestry. Three *PSENEN* mutations were not previously described: c.304T>A p.*102Argext*50 (patient 158), c.166+2T>C (patients 159 and 160), and c.66dup p.Phe23Valfs*98 (patient 161). Haplotype analysis for patients 159 and 160 did not reveal a common haplotype, arguing against a founder mutation (Figure 1). The c.166+2T>C mutation is predicted to abolish the splicing donor site (Supplementary Table S4). No causal mutation was identified in the remaining 160 patients.

Functional studies on primary keratinocytes from *PSENEN*-mutated patients 157 and 158 showed a 2-fold reduction in *PSENEN* transcript levels in patient 157 compared with controls and a 25% reduction in patient 158 (Supplementary Figure S2). Western blot analysis revealed no mutated PEN2 protein in both patients, a low level of wild-type protein (~13%) in patient 157 but a normal level of wild-type protein in patient 158 (Supplementary Figure S3). We could show impaired NCSTN maturation and stability of PSEN1 protein fragments and a reduction in *HES1* (Notch target) transcript levels in patient 157 compared with controls (Supplementary Figures S2 and S3), suggesting impaired gamma-secretase complex (GSC) function and Notch signaling. In contrast, patient 158 displayed no detectable

Abbreviations: GSC, gamma-secretase complex; HS, hidradenitis suppurativa

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Table 1. Patient Characteristics

Characteristic	n (%)
Sex	
Male	74 (44%)
Female	95 (56%)
Ethnicity	
Caucasian	107 (63%)
African	24 (14%)
Asian	3 (2%)
Other and mixed	23 (14%)
Unknown	12 (7%)
Form	
Familial	86 (51%)
Sporadic	48 (28%)
False sporadic[1]	27 (16%)
Unknown	8 (5%)
Hurley stage severity score	
1	62 (37%)
2	44 (26%)
3	56 (33%)
Unknown	7 (4%)
Body mass index (mean)	26.4 (range: 17.0–46.0)
<18.5	1 (<1%)
18.5–24.9	67 (40%)
25–29.9	54 (32%)
≥30	36 (21%)
Unknown	11 (6%)
Smoking history	115 (68%)
Age of HS onset (mean)	20 (range: 1–55 y)
Syndromic form	13 (8%)
PASH	5 (3%)
PAPASH	1 (<1%)
PASS	2 (1%)
HS + Dowling-Degos disease	5 (3%)
Comorbidities	
Severe acne	19 (11%)
Acne	34 (20%)
Dissecting cellulitis of the scalp	6 (4%)
Inflammatory bowel disease	18 (11%)
Inflammatory rheumatologic diseases	15 (9%)
Atopy, asthma, eczema	41 (24%)
Psoriasis	2 (1%)
Diabetes	14 (8%)
Adamantiades-Behçet's disease	1 (<1%)
Familial Mediterranean fever	1 (<1%)

Abbreviations: HS, Hidradenitis suppurativa; PAPASH, pyogenic arthritis, acne, pyoderma gangrenosum and suppurative hidradenitis; PASH, pyoderma gangrenosum, acne and suppurative hidradenitis; PASS, pyoderma gangrenosum, acne vulgaris, hidradenitis suppurativa, and ankylosing spondylitis.

There was a bias in the recruitment of patients with HS in this cohort because the enrollment mainly involved patients with severe forms of HS.

[1]False sporadic cases had in fact first- or second-degree relatives who presented either a few abscesses and/or cysts localized to HS predilection sites or isolated pilonidal sinus without completely fulfilling criteria for HS and/or had inflammatory bowel disease, rheumatologic inflammatory disease, or severe acne.

abnormality at the protein nor the RNA levels (Supplementary Figures S2 and S3), although previous studies reported the critical role of the length and sequence of the C-terminus of PEN2 for GSC activity (Prokop et al., 2005). Previous studies reported that some *NCSTN* mutations did not impair GSC activity nor Notch signaling (Zhang and Sisodia, 2015), that the *PSEN1* p.Pro242Leufs*11 mutation may lead to enhanced Notch signaling in zebrafish (Newman et al., 2014), and that the total number of mature GSC was unaltered in fibroblast solubilized membrane fractions harboring *NCSTN* p.Glu333_Gln367del or *PSENEN* p.Phe23Valfs*98 mutations (Pink et al., 2016) (Supplementary Table S2).

To date, Notch was used as a hallmark for GSC function, hypothesizing that Notch signaling was involved in HS pathogenesis. However, these findings suggest that the disease mechanisms are complex and that these mutations, together with the p.*102Argext*50 *PSENEN* mutation, may act through the alteration of a GSC catalytic activity independent mechanism and/or impaired Notch-independent downstream signaling. Alternatively, mutations may become deleterious only in particular cells or conditions such as temperature, pH, or salt concentrations. Therefore, the cells which were investigated (fibroblasts or epidermal keratinocytes) may not be appropriate and other cell types such as follicular keratinocytes or sebocytes could be more suitable to investigate HS pathophysiology. Further experiments to elucidate the functional consequences of these mutations on GSC activity and downstream pathways are required.

In summary, in this study as well as the previous ones, we have examined *GSC* gene mutations in a total of 188 patients with unrelated HS and identified mutations in 8 of 169 patients in the recent study and in 4 of 19 patients in our previous studies. Our findings confirm that *GSC* gene mutations are uncommon in the largest HS cohort investigated so far (12 mutations per 188 patients, 6.4%), further document *PSENEN* mutations in patients with HS with Dowling-Degos disease, report a European case of HS owing to a *PSEN1* mutation, and expand the spectrum of *NCSTN*-associated clinical manifestations.

Data availability statement

Targeted next-generation sequencing data sets related to this article are hosted at the National Center for Biotechnology Information Sequence Read Archive under the accession code PRJNA552677.

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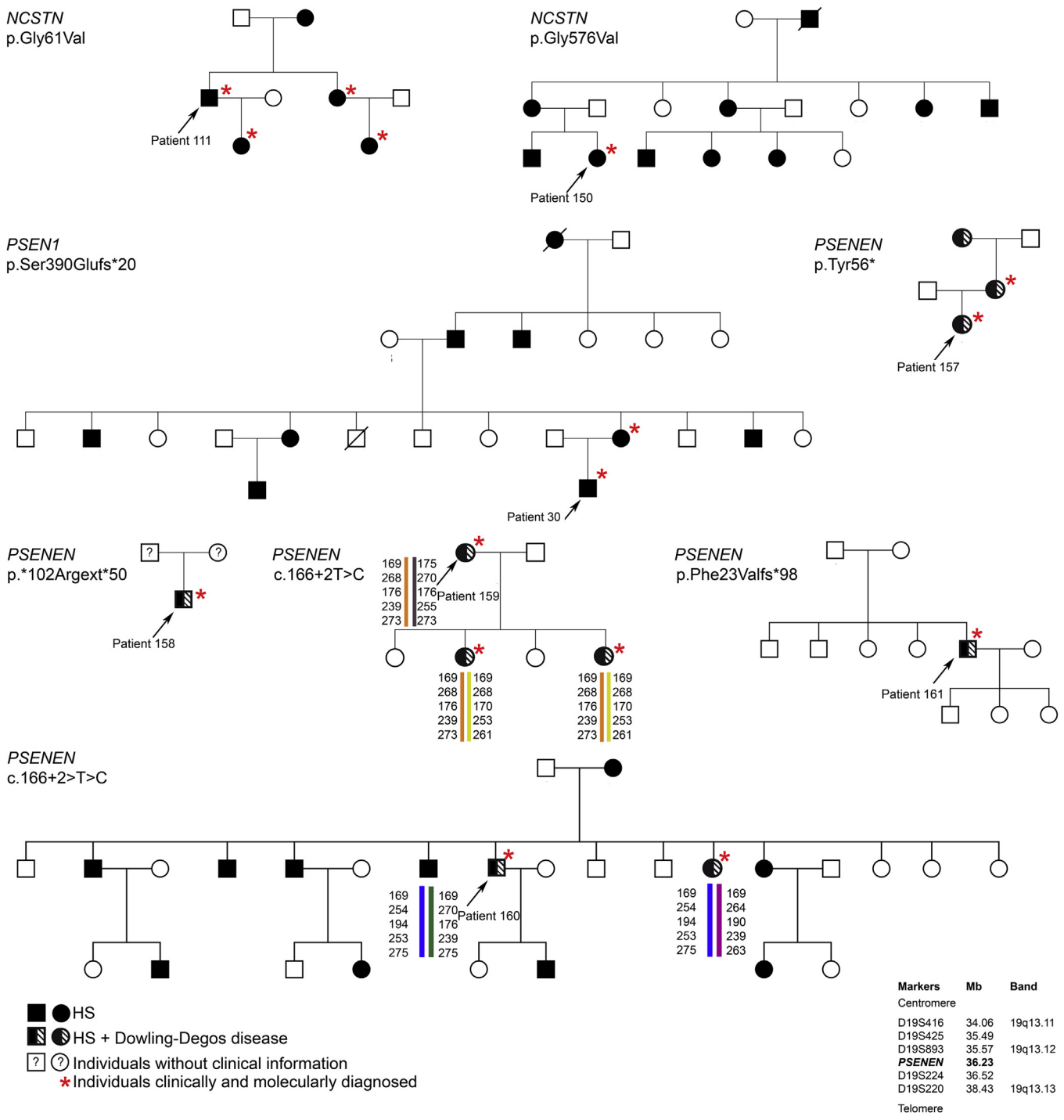

Figure 1. Genealogical trees of patients harboring mutations in *NCSTN*, *PSEN1*, or *PSENEN* genes. Arrows indicate the probands. Haplotype reconstruction with informative microsatellites markers D19S416, D19S425, D19S893, D19S224, and D19S220 on chromosome 19q13.11-q13.13 flanking *PSENEN* was performed for patient 159 and her two affected daughters and for patient 160 and one of his affected sisters, as illustrated. No common haplotype for these two families was identified. Patient 160 belongs to a family in which some patients presented only HS and were unavailable for the genetic study. Patient 2 *NCSTN* variant was excluded as there was no cosegregation of this splicing variant with HS and no effects on mRNA expression or splicing, arguing against a causal mutation. HS, Hidradenitis suppurativa.

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CONFLICT OF INTEREST

Outside of this publication, Véronique Del Marmol has received speaker and/or board fees from Bristol-Myers Squibb, AbbVie, and Sanofi. The remaining authors state no conflict of interest.

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AUTHOR CONTRIBUTIONS

Conceptualization: SD, AN, AH; Formal Analysis: SD; Funding Acquisition: OJL, AN, AH; Investigation: SD, SM; Methodology: SD, SH; Project Administration: AN, AH; Resources: MD, MNU, TL, FB, VDM, ARJVV, EPP, OC, MBB, CG, JV, OJL, MP, PN, SH, SF, HHVDZ, DB, GD, AA, YHL, GN, CCZ, AN; Supervision: AN, AH; Validation: SD, AN; Visualization: SD; Writing - Original Draft Preparation: SD; Writing - Review and Editing: SD, SM, MD, MNU, TL, FB, VDM, ARJVV, EPP, OC, MBB, CG, JV, OJL, MP, PN, SH, HHVDZ, DB, GD, AA, YHL, GN, CCZ, AN, AH.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2019.10.025.

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SUPPLEMENTARY MATERIALS AND METHODS

Clinical diagnostic criteria

Diagnosis of hidradenitis suppurativa was made according to European guidelines (Zouboulis et al., 2015a, 2015b), including recurrent or suppurating typical lesions (nodules, abscesses, sinus tracts, or hypertrophic scars) in typical localizations (axillae, inguinal folds, anal cleft, and submammary regions) occurring more than twice in six months.

Study subjects

The DNA bioresources originated from three protocols dedicated to studies of hidradenitis suppurativa, which were all approved by French Ethical Committee (CPP Ile-de-France I, CPP Ile-de-France 3). All protocols were parallelly approved by the French drug agency (AFFSSAPS/ANSM). Written informed consents were obtained from all included subjects. Samples and associated data were managed by the ICAReB platform at Pasteur Institute, Paris, France, or by Necker Imagine DNA biobank, Paris, France, following all applicable regulations and standards. The probands and their relatives provided blood samples. Patients 2, 157, and 158 provided punch biopsies of skin. De-identified normal skin from surgical margins was obtained for controls.

Targeted next-generation sequencing

A custom SureSelect gene panel was designed using the SureDesign software (*Homo sapiens*, hg19, GRCh37, February 2009, Agilent Technologies, Santa Clara, CA). The target regions covered 751.97 kb (panel v1) or 866.10 kb (panel v2), including coding exons and splice junctions of the 185 genes (panel v1: 2613 exons) or 210 genes (panel v2: 2803 exons), respectively. Illumina compatible precapture barecoded genomic DNA libraries were constructed according to the manufacturer's protocol (Ovation Ultralow, Nugen Technologies, Redwood city, CA). Briefly, genomic DNA (1−3 μg) was mechanically fragmented by sonication with a Covaris S2 Ultrasonicator (Covaris, Woburn, MA), ligated to multiplexing Illumina compatible paired-end adapters, amplified by PCR with indexed (barcoded) primers for sequencing, and hybridized to biotinylated complementary 120-bp RNA custom−designed capture probes in a solution-based reaction. Hybridization was performed at 65 °C for at least 16 hours, followed by paired-end sequencing on the Illumina HiSeq 2500 (130 × 130 bases) or on a NovaSeq 6000 (100 × 100 bases) at the Genomics Core Facility at the Imagine Institute.

Data analysis was performed with Paris Descartes University and/or Imagine Institute's Bioinformatics Core facility. After demultiplexing, sequences were aligned to the reference human genome hg19 using the Burrows-Wheeler Aligner. Downstream processing was carried out with the Genome Analysis Toolkit, SAMtools, and Picard, following documented best practices (http://www.broadinstitute.org/gatk/guide/topic?name=best-practices). Variant calls were made with the Genome Analysis Toolkit, Unified Genotyper, freebayes, and SAMtools on the basis of the 72nd version of ENSEMBL database. Variants were annotated and analyzed using the in-house PolyDiag software interface that filtered out irrelevant and common polymorphisms on the basis of frequencies extracted from public databases (dbSNP, Exome Variant Server, and Exome Aggregation Consortium). Consequences of mutations on protein function were predicted using Polyphen2 (http://genetics.bwh.harvard.edu/pph2), SIFT (https://sift.bii.a-star.edu.sg/), and MutationTaster (www.mutationtaster.org). Mutations were then ranked on the basis of the predicted impact of each variant by combined annotation-dependent depletion (Kircher et al., 2014) and compared with the mutation significance cutoff, a gene-level specific cutoff for combined annotation-dependent depletion scores (http://pec630.rockefeller.edu:8080/MSC) (Itan et al., 2016). To evaluate copy number variations (i.e., duplication and large deletion events for each individual), the relative read count for each targeted region was determined as the ratio of the read count for that region divided by the total absolute read counts of all targeted regions of the design. The ratio of the relative read count of a region in a given individual over the average relative read counts in other individuals of the run resulted in the estimated copy number for that region in that individual (method adapted from Goossens et al., 2009).

The mean depth of coverage per sample was >300× (except for patients 51, 148, 150, 152, and 155 for whom it comprised between 267× and 298×) allowing the maximization of detection of variant in regions with less coverage and to enable accurate copy number variant analysis of the exons of the panel. In average, 99% of the targeted exonic bases were covered at least with 30 independent reads.

Prioritization of the variants was performed by PolyDiag interface. We excluded known variants with a minor allele frequency ≥5% listed in databases or variants previously identified in in-house exomes. We subsequently selected for variants affecting splice sites or coding regions (nonsynonymous, nonsense, frameshift, and start and/or stop gain or loss) and predicted to be damaging by in silico prediction tools. Additional prediction tools were then used to predict the pathogenicity of candidate missense variants identified (Panther, MutationAssessor, AlignGVGD, SNAP2, SNP&GO, Condel, FATHMM, Provean, and MutPred2). Other computational tools were also used (VEST-4, MutPred-LOF, DDIG, and ENTPRISE-X). To evaluate the consequence of the splicing-site mutation, we used in silico prediction tools (SSF, MaxEnt, NSPLICE, GeneSplicer, and HSF).

Microsatellites genotyping

Primers sequences of microsatellite markers (available at the Working Draft of the Human Genome available at University of California Santa Cruz, Human Assembly [GRCh37/hg19]) used for haplotype analysis at *PSENEN* locus (19q13.12) are as follows;

D19S416 (AFM304XF5): Forward 5′-CCTGTCCCAGAGAGACCCTA-3′, Reverse 5′-AAGAGAGTGTGCCATTTGCT-3′;

D19S425 (AFMA139WE9): Forward 5′-CCACAGGTGTGCATAAAAG-3′; Reverse 5′-GCCATGTGACTGTAGCAGA-3′;

D19S893 (AFMB004WH1): Forward 5′-AATCCTGAGACTGGGGG-3′, Reverse 5′-TGGTGACACACTGGTGAC-3′;

D19S224 (AFM240VC1): Forward 5′-AACACCATTCCTCATCTTCC-3′, Reverse 5′-CCCAGGCCCTATCTGA-3′;

D19S220 (AFM214YF12): Forward 5′-ATGTTCAGAAAGGCCATGTCATTTG-3′, Reverse 5′-TCCCTAACGGATACACAGCAACAC-3′.

Fluorescein amidites–labeled amplified fragments were electrophoresed on an Applied Biosystems 3500XL genetic analyzer using GeneScan 400 HD ROX Size Standard (Life technologies, Carisbad, CA) and analyzed using the Gene-Mapper Software 5 (Applied Biosystems, Foster City, CA).

Cell culture

The 4-mm punch biopsies were explanted to isolate primary epidermal keratinocytes, which were maintained in Green medium on a feeder layer of lethally irradiated 3T3 mouse fibroblasts as described previously (Barrandon and Green, 1987). For experiments, keratinocytes were grown in 0.06 mM $CaCl_2$ EpiLife medium (Invitrogen, Carisbad, CA) to 70–80% confluence.

RNA isolation from primary human epidermal keratinocytes and RT-PCR

Subconfluent cultured keratinocytes were harvested, RNA was prepared through RNeasy Mini (Qiagen, Hilden, Germany), and cDNA was prepared with random hexamer priming and SuperScript IV reverse transcriptase (Life-Technologies). PCR was performed in triplicate for each sample with qPCR MesaGreen Mastermix (Eurogentec, Liège, Belgium) on a 7500 Sequence Detection (Applied Biosystems). Six control subjects were used, giving similar results. The experiment was replicated at least three times. PGK1 was used as an endogenous control.

RT-PCR primers

Quantitative PCR was performed with *PSENEN or HES1* and normalization to *PGK1*.

PSENEN: Forward 5′-ATGAACCTGGAGCGAGTGTC-3′, Reverse 5′-GTGTAGGCTGGGACAAGGAA-3′;

HES1: Forward 5′-CCAAAGACAG CATCTGAGCA-3′, Revrse 5′-AGAATG TCCGCCTTCTCCA-3′;

PGK1: Forward 5′-CTGTGGCTTC TGGCATACCT-3′, Reverse 5′-AATC TGCTTAGCCCGAGTGA-3′.

Protein isolation from primary human epidermal keratinocytes and immunoblotting

Keratinocytes were lysed in buffer (50 Mm, pH: 8, Tris-HCl, 150 mM NaCl; 1% Nonidet P-40, 5 Mm, pH: 8, EDTA pH: 8; Complete protease inhibitors [Roche, Basel, Switzerland]), incubated on ice for 30 minutes and sonicated for 2 minutes. Protein extracts were isolated by centrifugation at 13,000*g* at 4 °C for 20 minutes. 30 μg samples were loaded in Laemmli buffer (62.5 Mm, pH: 6.8, Tris-HCl, 5% β-mercaptoethanol, 2% SDS, 10% glycerol, 0.002% bromophenol blue), separated by SDS-PAGE, transferred onto Nitrocellulose membrane (Trans-Blot Turbo Nitrocellulose, Bio-Rad, Hercules, CA), and incubated with primary and secondary antibodies, with chemiluminescence detection (ECL Plus Western Blotting Substrate, Pierce) using the ChemiDoc Imaging System (Bio-Rad). β-Actin served as loading control. Experiments were replicated three times. Two control subjects were used, giving similar results.

Antibodies. Primary antibodies included rabbit polyclonal anti-PEN2 (Enzo ADI-905-736), rabbit polyclonal anti-NCSTN (N1660, Sigma, St. Louis, MO), and mouse monoclonal anti-PSEN1 (MAB5232, Chemicon International, Temecula, CA). Secondary antibodies were HRP-linked anti-rabbit or anti-mouse IgG antibodies (#7074 and #7076, Cell Signaling, Danvers, MA).

Statistical analyses. Statistical significance of observed differences was assessed by two-tailed Mann–Whitney *U* tests. Means with SEM are shown.

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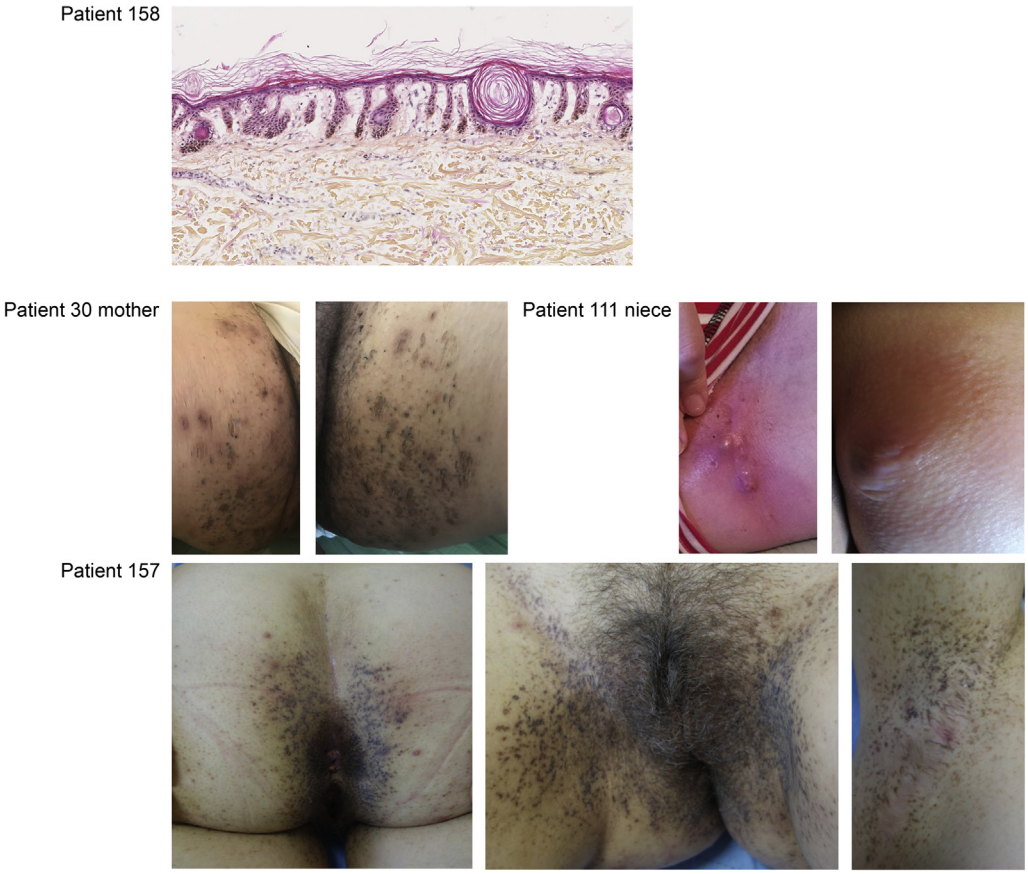
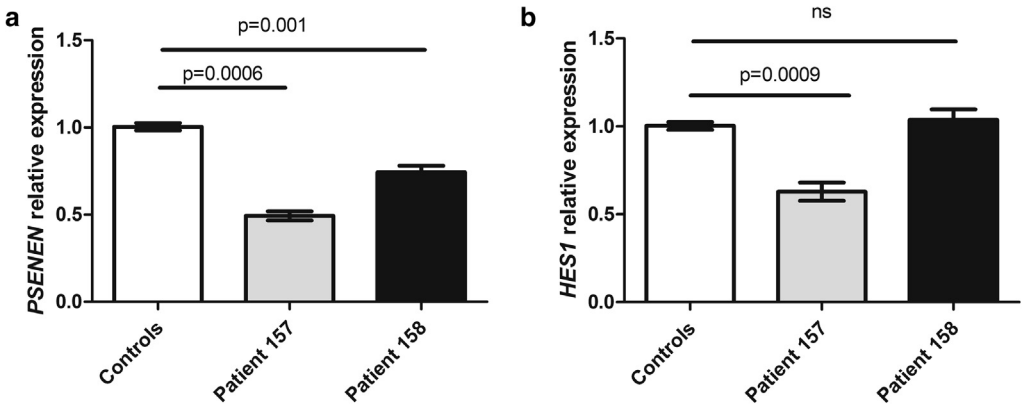
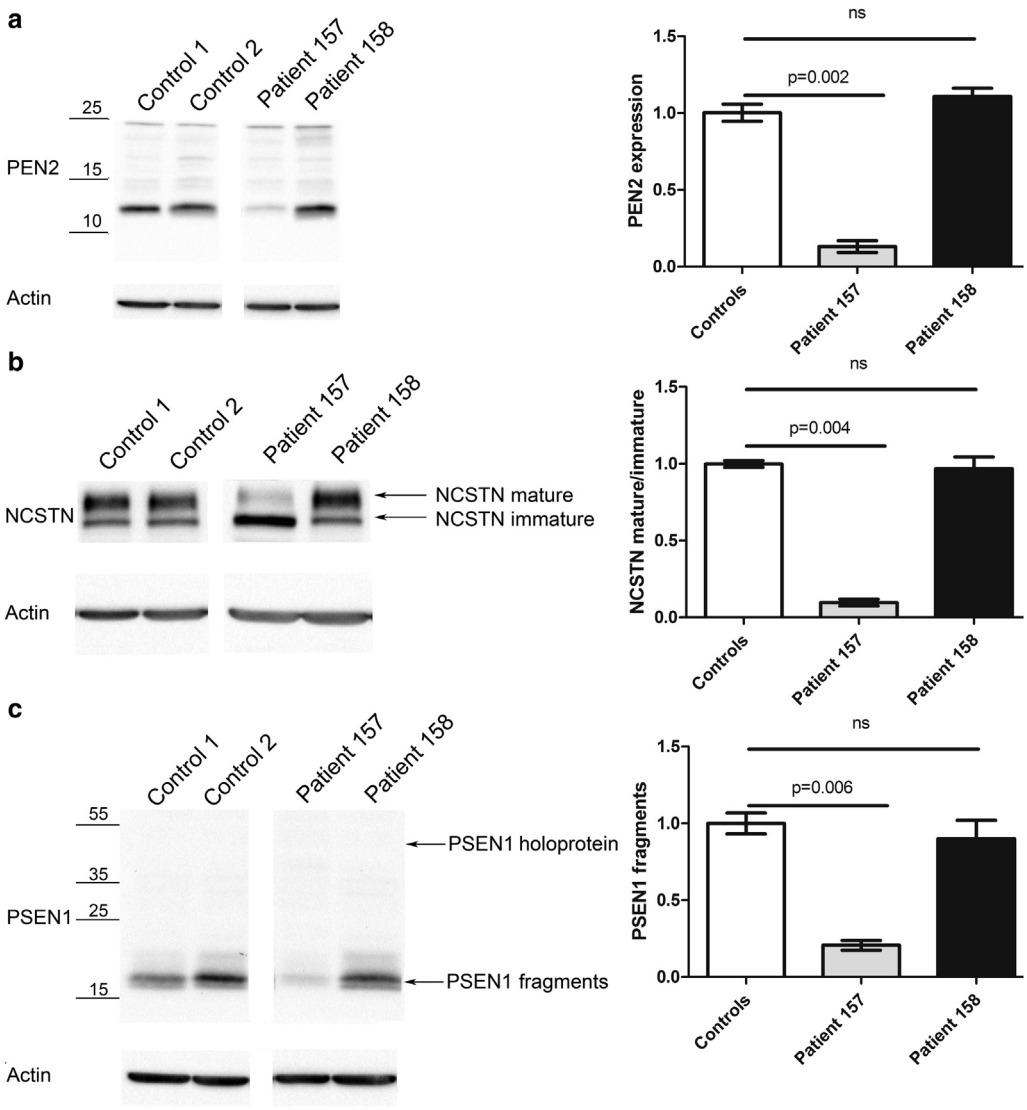


Supplemental Figure S1. Histopathological and clinical features of patients with HS with identified mutations. H&E staining of affected skin from patient 158 with HS and Dowling-Degos disease carrying a *PSENEN* mutation showed epidermal hyperplasia with elongated and hyperpigmented rete ridges. Hyperpigmentation and numerous multipore comedones in the groin of patient 30's affected mother (harboring *PSEN1* mutation and presenting with HS associated with Crohn's disease and ankylosing spondylitis). Nodules of the groin and the buttocks in patient 111's affected niece (carrying *NCSTN* mutation). Hyperpigmentation of the intergluteal fold, the groin, and the axillary regions in patient 157 (affected with HS and Dowling-Degos disease, harboring a *PSENEN* mutation). Subjects consented to the publication of their images. HS, Hidradenitis suppurativa.



Supplemental Figure S2. Relative *PSENEN* and *HES1* transcripts abundance in *PSENEN*-mutated patients versus control primary human epidermal keratinocytes. Quantitative RT-PCRs of keratinocyte RNA from patients 157 (p.Tyr56*) and 158 (p.*102Argext*50) show (**a**) decreased *PSENEN* expression level in both patients compared with control RNA, suggesting nonsense-mediated mRNA decay and unstable mutant mRNA degradation, respectively, and (**b**) reduction in *HES1* (used as a hallmark for Notch signaling activation) expression level in patient 157. *PGK1* was used as an endogenous control.



Supplemental Figure S3. Immunoblot of PEN2, NCSTN, and PSEN1 in *PSENEN*-mutated patients and control primary human epidermal keratinocytes. Western blot analysis of keratinocyte lysates from control subjects and patients 157 and 158 probed for (**a**) PEN2, (**b**) NCSTN, and (**c**) PSEN1 show decreased PEN2 levels, a significant reduction in mature NCSTN expression and in PSEN1 fragments in patient 157, but no abnormality for patient 158. No mutated PEN2 protein was observed in patients 157 and 158 (size prediction ~7 kDa for p.Tyr96* and ~17 kDa for p.*102Argext*50). Unaltered PSEN1 endoproteolysis was noted in both patients. Actin was used as a loading control.

Supplementary Table S1. Studies Investigating *GSC* Gene Mutations in Patients with HS

Reference	Number of Unrelated Patients Studied (Form)	Number of Patients with *NCSTN* Mutations (Form)	Number of Patients with *PSEN1* Mutations (Form)	Number of Patients with *PSENEN* Mutations (Form)	Ethnicity
Wang et al. (2010)	6 (Familial)	3 (Familial)	1 (Familial)	2 (Familial)	Chinese
Li et al. (2011)	2 (1 Familial + 1 Sporadic)	2 (1 Familial + 1 Sporadic)	—	—	Chinese
Liu et al. (2011)	2 (Familial)	2 (Familial)	—	—	Chinese
Pink et al. (2011)	7 (Familial)	1 (Familial)	0	1 (Familial)	British
Miskinyte et al. (2012)	14 (Familial)	3 (Familial)	0	0	French
Pink et al. (2012)	48 (20 Familial + 28 Sporadic)	3 (Sporadic)	0	0	British (2) and Afro-Caribbean (1)
Zhang et al. (2013)	2 (Familial)	2 (Familial)	—	—	Chinese
Ingram et al. (2013)	20 (12 Familial + 8 Sporadic)	0	0	0	South Wales
Jiao et al. (2013)	1 (Familial)	1 (Familial)	—	—	Chinese
Nomura et al. (2013)	10 (1 Familial + 9 Sporadic)	1 (Familial)			Japanese
Nomura et al. (2014)	1 (Familial)	1 (Familial)	—	—	Japanese
Chen et al. (2015)	1 (Familial)	1 (Familial)	—	—	African American
Duchatelet et al. (2015)	3 (2 Familial + 1 adopted child)	1 (Adopted child)	0	0	French
Yang et al. (2015)	1 (Familial)	1 (Familial)	—	—	Chinese
Panmontha et al. (2015)	2 (Familial)	—	—	2 (Familial)	Thai
Xu et al. (2016)	2 (Familial)	2 (Familial)	—	—	Chinese
Liu et al. (2016)	95 (57 Familial + 38 Sporadic)	2 (1 Familial + 1 Sporadic)	—	—	Australian, Canadian, Czech, Danish, French, German, Greeks, Hungarian, Dutch, Puerto Rican, Swedish, Swiss, Turkish, and American
Liu et al. (2016)	1 (Sporadic)	—	—	1 (Sporadic)	Chinese
Ratnamala et al. (2016)	2 (Familial)	2 (Familial)	—	—	Indian
Xiao et al. (2016)	1 (Familial)	1 (Familial)	—	—	Chinese
Faraji Zonooz et al. (2016)	1 (Familial)	1 (Familial)	—	—	Iranian
Zhang et al. (2016)	1 (Familial)	1 (Familial)	—	—	Chinese
Zhou et al. (2016)	2 (Familial)	—	—	2 (Familial)	Chinese
Pink et al. (2016)	1 (Familial)	1 (Familial)	—	—	British
Ralser et al. (2017)	6 (3 Familial + 3 Sporadic)	—	—	6 (3 Familial + 3 Sporadic)	German (3), French (1), Indian (1), Thai (1)
Li et al. (2017)	2 (Familial)	—	—	2 (Familial)	Chinese
Sonbol et al. (2018)	1 (Sporadic)	0	0	0	French
Pavlovsky et al. (2018)	4 (Familial)	—	—	4 (Familial)	Jewish Ashkenazi (4)
Kan et al. (2018)	1 (Familial)	—	—	1 (Familial)	Japanese
Wu et al. (2018)	1 (Familial)	1 (Familial)	—	—	Chinese
Shi et al. (2018)	1 (Familial)	1 (Familial)	—	—	Chinese
Xiao et al. (2018)	1 (Sporadic)	1 (Sporadic)	—	—	Chinese
Duchatelet et al. (2019)	1 (Familial)	0	0	0	French
He et al. 2019	3 (Familial)[1]	3 (Familial)[1]	—	—	Chinese
Takeichi et al. (2020)	1 (Familial)	1 (Familial)	—	—	Japanese

Abbreviation: HS, Hidradenitis suppurativa.

[1]In total, five familial HS cases harboring *NCSTN* mutations were reported, but two of them were previously published in Liu et al., 2011 and Xiao et al., 2016.

Supplementary Table S2. Summary of *GSC* Gene Mutations Reported in HS

Gene	Mutation		Familial Segregation	Functional Studies	Reference
	Nucleotide	Protein			
NCSTN	c.97G>A	p.Gly33Arg	Yes	None	Takeichi et al. (2020)
	c.210_211delAG	p.Val72Tyrfs*16[1]	Yes	None	Liu et al. (2011)
	c.218delC	p.Ile73Thrfs*3[2]	Yes	None	Wu et al. (2018)
	c.223G>A	p.Val75Ile	Yes	In vitro assay in fibroblasts: no impact on PSEN1 endoproteolysis, Notch processing, nor nuclear signaling	Zhang et al. (2013) / Zhang and Sisodia (2015)
	c.344_351del[3]	p.Thr115Asnfs*20	N/A (adopted child)	None	Duchatelet et al. (2015)
	c.349C>T	p.Arg117*	Yes/Yes/NR (familial)	Blood mRNA study: reduced *NCSTN* expression	Wang et al. (2010)/Chen et al.(2015)/Liu et al. (2016)
	c.477C>A	p.Cys159*	Yes	Skin immunohistochemistry: reduced NCSTN staining, Skin mRNA, and protein study; decreased NSCTN expression	Xiao et al. (2016) / He et al. (2019)
	c.487delC	p.Gln163Serfs*39	Yes	mRNA study from peripheral blood mononuclear cells: significant reduction of transcript level	Miskinyte et al. (2012)
	c.553G>A	p.Asp185Asn	NR (sporadic)	In vitro assay in fibroblasts: no impact on PSEN1 endoproteolysis, Notch processing, nor nuclear signaling.	Pink et al. (2012) / Zhang and Sisodia (2015)
	c.582+1delG	p.Cys195Lysfs*16 (exon 6 skipping) + p.Cys195_Gly332del (exons 6-8 skipping)	Yes	Peripheral lymphocytes mRNA study: marked reduction of *NCSTN* expression and abnormal splicing	Nomura et al. (2013)
	c.617C>A	p.Ser206*	Yes	None	Shi et al. (2018)
NCSTN	c.632C>G	p.Pro211Arg	Mutation not found in the patient's healthy relatives (sporadic)	In vitro assay in fibroblasts: no impact on PSEN1 endoproteolysis, Notch processing, nor nuclear signaling	Li et al. (2011) / Zhang and Sisodia (2015)
	c.647A>C	p.Gln216Pro	Yes	In vitro assay in fibroblasts: functionally inactive mutant (on Notch signaling processing and PSEN1 endoproteolysis) but effect on activity negated when the mutant is coexpressed with the wild-type	Zhang et al. (2013) / Zhang and Sisodia (2015)
	c.686_687dup[4]	p.Cys230Profs*32[4]	Yes	None	Ratnamala et al. (2016)
	c.887A>G	p.Glu296Gly	Yes	None	Xu et al. (2016)
	c.944C>T	p.Ala315Val	Yes	None	Zhang et al. (2016)
	c.996+1G>A[5]	p.Glu333_Gln367del (exon 9 skipping)	NR	Fibroblast mRNA study: abnormal transcript, reduced *NCSTN* expression; Fibroblast protein study: no mutant protein detected, decreased mature NCSTN in total cell fractions but no abnormality for NCSTN, PSEN1, and PEN2 expression in solubilized membrane preparations	Pink et al. (2016)
	c.996+7G>A[6]	p.?	NR (sporadic)	Lymphoblast mRNA study: reduced *NCSTN* expression	Pink et al. (2012)
	c.1101+1 G>A	No abnormal transcript	Yes	mRNA study: No abnormal transcript detected, reduced *NCSTN* expression	Pink et al. (2011)
	c.1101+10 A>G[7]	p.?	NR (sporadic)	Lymphoblast mRNA study: no aberrant transcripts, normal *NCSTN* expression	Pink et al. (2012)
	c.1229C>T	p.Ala410Val	NR(sporadic)	None	Liu et al. (2016)

Supplementary Table S2. Continued

Gene	Mutation		Familial Segregation	Functional Studies	Reference
	Nucleotide	Protein			
NCSTN	c.1258C>T	p.Gln420*	Yes	mRNA and protein studies from whole skin, epidermis, dermis, keratinocytes, or fibroblasts: reduced expression	Jiao et al. (2013)/Yang et al. (2015)
	c.1294C>T	p.Arg432*	Yes	Skin immunohistochemistry: reduced NCSTN staining; Skin mRNA and protein study: decreased NSCTN expression	He et al. (2019)
	c.1300C>T	p.Arg434*	Yes/Yes	mRNA study from peripheral blood mononuclear cells: normal expression	Miskinyte et al. (2012) / Xu et al. (2016)
	c.1352+1G>A	p.?	Yes/Yes	Skin immunohistochemistry: reduced NCSTN staining; Skin mRNA and protein study: decreased NSCTN expression	Liu et al. (2011) / He et al. (2019)
	c.1534C>T	p.Gln512*	Yes	Skin immunohistochemistry: reduced NCSTN staining; Skin mRNA and protein study: decreased NSCTN expression	He et al. (2019)
	c.1551+1G>A	p.Ala486_Thr517del	Yes	Blood mRNA study: Reduced *NCSTN* expression; abnormal splicing with exon 13 skipping	Wang et al. (2010)
	c.1635C>G[3]	p.Tyr545*	Yes	None	Faraji Zonooz et al. (2016)
	c.1695T>G	p.Tyr565*	Yes	None	Li et al. (2011)
	c.1702C>T	p.Gln568*	Yes (incomplete penetrance)	None	Nomura et al. (2014)
	c.1752delG	p.Glu584Aspfs*44	Yes	Blood and skin mRNA study: reduced *NCSTN* expression; Skin protein study: decreased NSCTN expression; Skin immunohistochemistry: reduced NCSTN staining	Wang et al. (2010) / He et al. (2019)
	c.1768 A>G	p.Ser590AlafsX3 and another product	Yes	mRNA study from peripheral blood mononuclear cells: abnormal splicing; no reduction of *NCSTN* transcript levels	Miskinyte et al. (2012)
NCSTN	c.1800_1801delTG[8]	p.Tyr600*[8]	Yes	None	Ratnamala et al. (2016)
	c.2584-2585delCA	Reduced expression	NR (sporadic)	Reporter assay in HEK293T cells: decreased expression through miR-155	Xiao et al. (2018)
PSEN1	c.725delC	p.Pro242Leufs*11	Yes	Blood mRNA study: reduced *PSEN1* expression; Zebrafish study: increased Notch signaling	Wang et al. (2010) / Newman et al. (2014)
	c.953A>G[9]	p.Glu318Gly	NR	None	Ingram et al. (2013)
PSENEN	c.35T>A[10]	p.Leu12*	Yes	None	Ralser et al. (2017)
	c.43_56del[10]	p.Cys15Profs*101[11]	Yes	None	Kan et al. (2018)
	c.62-1G>C[12]	p.Gly22_Tyr56del (exon 3 skipping)	Yes	Exon trapping: exon 3 skipping	Ralser et al. (2017)
	c.66delG[10]	p.Phe23Leufs*46	Yes/De novo mutation (sporadic)	None	Wang et al. 2010 / Liu et al. (2016)
	c.66_67insG[10]	p.Phe23Valfs*98[11]	Yes	Blood and fibroblast mRNA study: reduced *PSENEN* expression; Fibroblast protein study: no mutant protein detected, reduced PEN2 expression in total cell fractions but no abnormality for NCSTN, PSEN1, and PEN2 expression in solubilized membrane preparations.	Pink et al. (2011) / Pink et al. (2016)
	c.84dup	p.Leu29Serfs*92[11]	Yes	Leukocyte mRNA study: Increased *PSENEN* expression	Panmontha et al. (2015)
	c.115C>T[10]	p.Arg39*	NR (sporadic)	Protein expression in HEK293T cells: no mutant protein	Ralser et al. (2017)
	c.166+2T>C[10,13]	p.Gly22_Tyr56del (exon 3 skipping)	NR (sporadic)	mRNA study: abnormal splicing	Ralser et al. (2017)
	c.167-2A>G[10]	p.?	Yes	None	Zhou et al. (2016)

(*continued*)

Supplementary Table S2. Continued

Gene	Mutation		Familial Segregation	Functional Studies	Reference
	Nucleotide	Protein			
PSENEN	c.168T>G[10]	p.Tyr56*	Yes	mRNA study from keratinocytes: decreased *PSENEN* expression; Notch-responsive reporter assay in primary keratinocytes: decreased Luciferase activity	Pavlovsky et al. (2018)
	c.194T>G[10]	p.Leu65Arg	Yes/Yes	None	Zhou et al. (2016) / Li et al. (2017)
	c.229_230insCACC[10]	p.Ile77Thrfs*45[11]	Yes	None	Li et al. (2017) (Zhang et al. unpublished data)
	c.279delC	p.Phe94Serfs*51[11]	Yes	None	Wang et al. (2010)

Abbreviations: HS, Hidradenitis suppurativa; PASH, pyoderma gangrenosum, acne, and suppurative hidradenitis; N/A, not applicable; NR, not reported.

Mutations are numbered according to the nomenclature of the Human Genome Variation Society, such that +1 is the A of the start codon (ATG) of the cDNA sequences of *NCSTN* (Genbank accession number NM_015331), *PSEN1* (NM_000021), and *PSENEN* (NM_172341).

[1]This mutation was initially reported as p.Thr70fsX18.

[2]This mutation was initially reported as p.Pro73Leufs*15.

[3]PASH syndrome.

[4]This mutation was initially reported as c.687insCC p.Cys230Profs*31.

[5]This mutation was initially reported as c.1125+1G>A.

[6]This variant is unlikely to be causal according to our findings (absence of cosegregation with the disease and no effect on *NCSTN* splicing or expression).

[7]This variant was reported to be unlikely pathogenic on the basis of mRNA study results.

[8]This mutation was initially reported as c.1799delTG p.Leu600X.

[9]These variants were reported to be unlikely pathogenic (absence of cosegregation with the disease, observed in healthy controls, predicted to be benign).

[10]HS associated with Dowling-Degos disease or hyperpigmentation.

[11]Mutations predicting an altered and elongated protein.

[12]Patients with this mutation presented Dowling-Degos disease associated or not with HS.

[13]This mutation was initially reported as g.1412T>C.

Supplementary Table S3. Clinical Information for the 169 Patients with HS from the Cohort

Patient Number	Form	Sex	Geographic Origin	Age	BMI	Smoking History	Age at Onset	Hurley Stage	Comorbidities
1	Familial	F	EU	40	20.2	Yes	18	1	Atopy
2	False sporadic	M	EU	27	20.9	Yes	18	1	Acne, atopy
3	Familial	M	EU	28	20.6	Yes	13	1	DCS, eczema
4	False sporadic	F	EU	51	29.0	Yes	25	1	Atopy
5	Familial	F	EU	46	30.2	Yes	27	1	Atopy
6	Familial	F	EU	30	34.9	Yes	13	1	Acne, atopy
7	Familial	F	EU	47	26.7	Yes	15	1	Severe acne, atopy, AS
8	Familial	F	AF	26	35.9	Yes	15	1	atopy, SAPHO syndrome
9	Familial	F	AF	27	21.9	No	7	1	Atopy
10	Sporadic	F	EU	27	29.0	Yes	19	1	Acne, atopy
11	Familial	F	EU	49	26.8	Yes	19	1	Acne, atopy
12	Familial	F	EU	38	27.0	Yes	19	1	Acne, atopy
13	Familial	F	EU	31	26.0	Yes	13	2	Diabetes, atopy
14	Familial	M	AF	37	21.6	Yes	18	2	Acne, atopy
15	Familial	M	AF	30	21.8	No	18	2	Acne
16	Sporadic	F	EU	47	23.9	Yes	24	2	None
17	Sporadic	M	EU	47	31.4	Yes	24	2	Severe acne, atopy
18	Sporadic	F	EU	35	28.1	Yes	10	2	Acne, atopy
19	False sporadic	M	EU	35	25.0	Yes	24	2	Acne, atopy
20	Familial	M	EU	26	22.0	Yes	12	2	Acne, atopy
21	Sporadic	M	EU	31	21.0	Yes	24	2	Acne, atopy
22	Sporadic	M	EU	49	20.6	Yes	12	2	Severe acne, atopy
23	Familial	M	EU	28	27.8	Yes	17	2	Severe acne, atopy
24	Familial	M	AF	58	27.5	Yes	18	2	Diabetes, atopy, acne
25	Sporadic	M	EU	32	20.1	Yes	17	2	None
26	Familial	F	EU	34	35.0	Yes	16	2	SAPHO syndrome, atopy
27	Familial	F	EU	33	25.3	Yes	14	2	fibromyalgia
28	Familial	F	EU	39	31.2	No	13	2	Gougerot-Sjogren syndrome, atopy
29	Familial	F	EU	43	20.1	Yes	21	2	Chronic diarrhea
30	Familial	M	EU	25	32.0	Yes	17	1	Crohn's disease, atopy
31	Familial	F	EU	48	33.0	Yes	29	1	Craniopharyngioma and adrenal insuffisiency, diabetes
32	Sporadic	M	EU	24	27.0	No	15	2	None
33	Sporadic	F	EU	31	21.3	Yes	14	1	Unlabeled arthralgias, acne
34	Sporadic	M	AF	28	17.0	No	19	3	DCS, eczema
35	Sporadic	M	EU	38	24.5	Yes	22	1	Severe acne, atopy
36	Sporadic	F	AF	35	25.0	No	16	3	Breast cancer
37	Familial	F	AF	29	28.4	Yes	21	1	Atopy, osteomalacia
38	Familial	F	AF	55	20.0	No	16	1	Systemic Lupus
39	Sporadic	M	EU	41	27.0	Yes	22	2	DCS
40	False sporadic	M	EU	19	28.2	No	11	1	Acne
41	Sporadic	M	EU-Maghreb	34	22.2	Yes	26	1	Irritable bowel syndrome, asthma
42	False sporadic	M	EU	29	24.9	Yes	25	2	None
43	Sporadic	F	EU	36	28.1	Yes	17	1	AS, severe acne
44	Sporadic	F	AF	38	33.9	Yes	17	2	diabetes
45	False sporadic	F	EU	36	31.6	No	27	3	Acne
46	False sporadic	F	EU	49	28.9	Yes	27	2	Atopy
47	False sporadic	F	EU	25	19.5	No	19	2	Crohn's disease, acne, atopy
48	False sporadic	F	EU	41	25.2	Yes	30	3	Crohn's disease
49	False sporadic	F	EU	20	22.9	No	14	1	None
50	Familial	F	Saudi Arabia	27	39.3	No	9	3	Anodontia
51	Familial	M	Maghreb	29	27.8	Yes	15	2	Acne
52	Familial	F	n/a	20	N/A	N/A	16	3	N/A
53	Familial	M	EU	61	26.1	Yes	25	3	None
54	Familial	M	EU	62	20.3	Yes	40	3	None
55	Familial	M	EU	32	28.9	Yes	23	2	Severe acne

(*continued*)

Supplementary Table S3. Continued

Patient Number	Form	Sex	Geographic Origin	Age	BMI	Smoking History	Age at Onset	Hurley Stage	Comorbidities
56	Familial	M	EU	25	29.4	No	21	3	Severe acne, atopy
57	Familial	M	EU	35	21.6	No	13	1	Severe acne, atopy
58	Familial	M	EU	38	31.8	Yes	22	2	DCS
59	Familial	F	EU	41	31.6	Yes	15	1	Darier's disease
60	Familial	M	Maghreb	27	24.8	Yes	9	1	None
61	Familial	M	EU	30	29.4	Yes	16	2	None
62	Familial	F	EU	N/A	N/A	N/A	N/A	N/A	None
63	Familial	M	AF-EU	40	20.2	Yes	17	2	Crohn's disease, eczema
64	Familial	M	AF	63	31.0	No	33	3	diabetes, acne
65	False sporadic	M	EU	33	23.5	Yes	25	3	Aevere acne
66	False sporadic	M	EU	42	29.0	Yes	26	3	Aevere acne, diabetes, eczema
67	Familial	F	EU	44	28.3	Yes	15	3	Atopy
68	N/A	M	EU	69	24.2	Yes	52	3	Aevere acne, DCS
69	Familial	M	EU	71	30.9	Yes	55	2	Diabetes, atopy
70	Sporadic	M	EU	38	22.9	Yes	17	1	severe acne
71	Familial	F	EU	36	20.0	Yes	24	2	Crohn's disease
72	false Sporadic	F	N/A	34	N/A	N/A	15	3	Plantar keratoderma, multiple sclerosis
73	Familial	M	AF	29	19.4	Yes	18	3	Crohn's disease
74	False sporadic	F	EU	31	20.1	Yes	16	2	AS
75	Sporadic	M	N/A	N/A	N/A	N/A	N/A	N/A	DCS
76	Familial	F	AF	31	23.6	No	16	3	Breast cancer
77	Familial	F	AF	24	36.1	No	17	2	Epidermolysis bullosa, atopy, eczema
78	Sporadic	M	AF	25	27.5	Yes	17	3	Severe acne
79	Familial	M	Maghreb	62	23.9	Yes	34	3	Acne
80	False sporadic	F	EU	38	29.5	Yes	12	3	Acne
81	False sporadic	M	EU	38	29.5	Yes	31	3	Acne, atopy
82	Familial	M	EU	35	22.6	Yes	15	1	Acne
83	Familial	M	EU	52	25.7	Yes	17	3	Aone
84	Familial	F	EU	40	27.7	Yes	15	1	RA, atopy, psoriasis
85	False sporadic	F	EU	50	21.3	Yes	25	3	Crohn's disease, RA, Behçet's disease, APL syndrome
86	Sporadic	F	AF-EU	64	23.4	No	54	2	Crohn's disease, Steinert myotonia, diabetes
87	Familial	F	EU	45	32.5	Yes	15	1	None
88	Familial	F	EU	42	25.3	Yes	18	1	AS
89	N/A	F	Maghreb	32	19.9	Yes	11	1	None
90	False sporadic	F	EU	35	24.4	Yes	20	1	None
91	Familial	F	EU	52	21.0	Yes	30	1	Acne
92	Familial	F	EU	37	23.7	Yes	15	1	Ulcerative colitis
93	Familial	M	EU-Maghreb	35	25.5	Yes	23	1	None
94	Familial	F	EU	35	21.0	Yes	20	1	None
95	False sporadic	F	EU	49	27.4	Yes	17	1	Acne
96	N/A	F	AF	35	33.5	Yes	16	1	Acne
97	Familial	M	Asia	48	32.6	No	15	1	Diabetes, acne
98	Familial	F	EU	56	24.7	Yes	18	1	None
99	Familial	M	AF	31	21.9	No	19	2	None
100	Familial	M	EU	55	29.9	Yes	46	2	None
101	False sporadic	F	EU	39	28.7	Yes	28	2	AS
102	Sporadic	F	Maghreb	25	20.6	Yes	15	3	SAPHO syndrome
103	Familial	M	EU	15	28.0	No	13	3	None
104	Familial	M	AF	13	22.6	No	8	3	None
105	Familial	F	EU	30	27.6	Yes	22	3	Crohn's disease
106	Familial	F	AF	19	26.3	No	11	3	Adrenal hyperplasia
107	Familial	M	EU	16	21.8	No	13	2	Severe acne, craniostenosis
108	Familial	F	EU	25	23.9	No	11	3	None
109	False sporadic	M	EU	46	33.0	Yes	20	3	Diabetes

(*continued*)

Supplementary Table S3. Continued

Patient Number	Form	Sex	Geographic Origin	Age	BMI	Smoking History	Age at Onset	Hurley Stage	Comorbidities
110	Familial	M	EU	29	28.4	N/A	N/A	3	FMF
111	Familial	M	EU	51	32.7	No	17	1	None
112	Sporadic	M	EU	56	36.8	Yes	13	3	Diabetes
113	Familial	F	N/A	26	N/A	N/A	N/A	N/A	None
114	Sporadic	F	AF	30	27.5	No	15	3	None
115	Familial	F	EU	40	21.5	No	19	2	Crohn's disease, AS, PG
116	Sporadic	F	N/A	33	N/A	n/a	31	3	None
117	Familial	F	EU-AF	24	23.2	Yes	17	3	Severe acne, severe acne
118	Sporadic	M	EU	35	20.0	Yes	16	3	None
119	Sporadic	M	EU	44	21.9	No	12	3	Crohn's disease
120	N/A	F	Maghreb	48	22.0	No	47	3	None
121	Familial	F	AF	31	33.4	Yes	11	3	AS
122	Sporadic	F	AF	23	18.8	No	11	2	Crohn's disease
123	Familial	F	EU	21	23.1	Yes	13	2	Ulcerative colitis
124	Familial	F	Asia	17	20.7	No	16	3	Severe acne
125	Sporadic	F	AF	46	23.5	Yes	19	1	None
126	Sporadic	M	N/A	29	30	Yes	20	1	Severe acne
127	Sporadic	F	N/A	36	N/A	Yes	N/A	1	N/A
128	Sporadic	F	N/A	33	N/A	No	11	1	None
129	Familial	M	N/A	33	32.4	Yes	N/A	1	None
130	Familial	F	EU	71	27.1	Yes	22	1	None
131	Familial	F	EU	38	39.0	Yes	20	1	Crohn's disease
132	N/A	F	Iran	24	26.2	Yes	6	1	None
133	Familial	M	Maghreb	37	34.3	Yes	21	1	Diabetes
134	Sporadic	F	EU	42	35.5	Yes	16	1	None
135	Familial	F	EU	33	28.9	Yes	20	1	None
136	Sporadic	F	Maghreb	31	35.5	No	22	1	None
137	Sporadic	F	AF	42	35.6	No	32	1	None
138	Sporadic	F	EU+AF	23	27.6	Yes	13	1	None
139	Sporadic	F	EU	28	22.1	Yes	22	1	Severe acne
140	Sporadic	F	EU	42	27.7	Yes	20	1	None
141	Sporadic	F	EU	28	30.1	Yes	11	1	None
142	N/A	F	EU	33	28.6	Yes	18	1	None
143	Sporadic	F	EU	25	22.3	Yes	19	1	None
144	N/A	M	EU	29	24.8	Yes	15	3	None
145	false Sporadic	F	EU	26	23.2	Yes	15	3	None
146	Sporadic	F	Saudi Arabia	16	43.0	No	1	3	None
147	Sporadic	F	EU	25	21.7	Yes	15	3	None
148	Sporadic	M	EU	44	27.4	Yes	27	3	None
149	false Sporadic	M	Maghreb	71	22.7	Yes	40	3	None
150	Familial	F	EU	30	21.7	No	23	3	None
151	false Sporadic	M	n/a	30	n/a	Yes	26	1	None
152	Sporadic	F	Algeria	58	31.2	Yes	50	3	Psoriasis
153	false Sporadic	M	EU	34	26.3	Yes	23	2	None
154	Familial	F	EU	47	25.1	No	22	3	AS
155	Familial	F	Maghreb	50	33.2	No	38	3	Diabetes
156	false Sporadic	M	EU	29	23.1	Yes	22	3	Crohn's disease
157	Familial	F	EU	44	21.4	No	24	1	Dowling-Degos
158	N/A	M	EU	60	26.2	No	18	3	Dowling-Degos, diabetes, atopy
159	Familial	F	EU	66	25.4	No	13	N/A	Dowling-Degos
160	Familial	M	EU	54	42.4	N/A	15	N/A	Dowling-Degos
161	Sporadic	M	N/A	53	N/A	N/A	N/A	N/A	Dowling-Degos
162	Familial	F	EU	56	27	Yes	37	3	PG+Acne: PASH syndrome
163	Familial	M	Carabbean	35	19.9	Yes	30	3	PG+Acne: PASH syndrome
164	False sporadic	M	EU-Algeria-Morocco	22	19.6	Yes	14	3	PG+Acne+Spondyloarthropathy: PASS syndrome
165	Sporadic	M	Morocco	47	27.2	Yes	n/a	2	PG+Acne: PASH syndrome

(*continued*)

Supplementary Table S3. Continued

Patient Number	Form	Sex	Geographic Origin	Age	BMI	Smoking History	Age at Onset	Hurley Stage	Comorbidities
166	Sporadic	F	EU	40	20.8	Yes	18	3	PG+Acne+arthritis: PAPASH syndrome
167	Sporadic	M	EU	45	32.4	No	16	2	PG+Acne+Spondyloarthropathy: PASS syndrome
168	Familial	M	n/a	22	n/a	n/a	n/a	n/a	PG+Acne: PASH syndrome
169	Sporadic	M	Asia	40	22.1	No	35	2	PG+Acne: PASH syndrome

Abbreviations: AF, Africa; APL, antiphospholipid; AS, Ankylosing Spondylarthritis; BMI, Body Mass Index; DCS, Dissecting cellulitis of the scalp; EU, Europe; FMF, Familial Mediterranean fever; HS, Hidradenitis suppurativa; N/A, not applicable; PAPASH, pyogenic arthritis, acne, pyoderma gangrenosum, and suppurative hidradenitis; PASH, pyoderma gangrenosum, acne, and suppurative hidradenitis; PASS, pyoderma gangrenosum, acne vulgaris, hidradenitis suppurativa, and ankylosing spondylitis; PG, Pyoderma gangrenosum; RA, Rheumatoid arthritis; SAPHO, Synovitis, acne, pustulosis palmoplantaris, hyperostosis, and osteitis.

Supplementary Table S4. In Silico Prediction Analysis of Identified Mutations

Gene	Mutation	Grantham Score	Polyphen-2	SIFT	MutationTaster	Panther	Mutation Assessor	AlignGVGD	SNAP2
NCSTN	p.Gly576Val	109	Probably damaging (score: 0.998)	Deleterious (score: 0.05)	Disease causing (prob: 0.999)	Probably damaging (preservation time: 1368)	Impact function: medium (FI Score: 2.745)	Most likely to interfere with function (Class 65)	Effect (score: 81)
NCSTN	p.Gly61Val	109	Probably damaging (score: 0.999)	Deleterious (score: 0.00)	Disease causing (prob: 0.999)	Probably damaging (preservation time: 1628)	Impact function: medium (FI Score: 3.085)	Most likely to interfere with function (Class 65)	Effect (score 93)
PSEN1	p.Ser390Glufs*20	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
PSENEN	p.Tyr56*	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
PSENEN	p.*102Argext*50	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
PSENEN	p.Phe23Valfs*98	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Gene	Mutation	SNP&GO	Condel	FATHMM	Provean	MutPred2	VEST-4	MutPred-LOF	DDIG	ENTPRISE-X
NCSTN	p.Gly576Val	Disease (prob: 0.759)	Deleterious (score 0.632)	Pathogenic (score: -1.56)	Deleterious (score: -4.78)	Functional consequences (score: 0.890)	Pathogenic (score: 0.955)	N/A	N/A	N/A
NCSTN	p.Gly61Val	Disease (prob: 0.913)	Deleterious (score: 0.656)	Pathogenic (score: -5.29)	Deleterious (score -8.50)	Functional consequences (score: 0.931)	Pathogenic (score: 0.997)	N/A	N/A	N/A
PSEN1	p.Ser390Glufs*20	N/A	N/A	N/A	N/A	N/A	Pathogenic (score: 0.971)	No pathogenicity (score: 0.445)	Disease (score: 0.935)	Deleterious (score: 0.970)
PSENEN	p.Tyr56*	N/A	N/A	N/A	N/A	N/A	Pathogenic (score: 0.887)	Pathogenicity (score: 0.548)	Disease (score: 0.842)	Deleterious (score: 0.973)
PSENEN	p.*102Argext*50	N/A	N/A	N/A	N/A	N/A	Benign (score: 0.088)	No pathogenicity (score: 0.380)	N/A	N/A
PSENEN	p.Phe23Valfs*98	N/A	N/A	N/A	N/A	N/A	Pathogenic (score: 0.853)	No pathogenicity (score: 0.439)	Disease (score: 0.883)	Deleterious (score: 0.893)

Gene	Mutation	SSF	MaxEnt	NNSPLICE	Gene Splicer	HSF
PSENEN	c.166+2T>C	Loss of donor site (71.05 ⇒ -)	Loss of donor site (7.39 ⇒ -)	Loss of donor site (0.72 ⇒ -)	Loss of donor site (4.70 ⇒ -)	Loss of donor site (81.46 ⇒ 54.63)

Abbreviations: FI, functional impact; N/A, not applicable; prob, probability.